Section 1. General administrative information

Evaluate Feed Strategies to Reduce Residualism and Promote Smolting in Steelhead

Bonneville project number, if an ongoing project 9082

Business name of agency, institution or organization requesting funding

Idaho Fishery Resource Office, U.S. Fish and Wildlife Service with joint sponsors.

Business acronym (if appropriate) IFRO-USFWS

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Subcontractors.

Joint Sponsors list: Individual budgets for joint sponsors are available upon request.

Organization	Mailing Address	City, ST Zip	Contact Name
USGS-BRD, Columbia River Research Laboratory	5501A Cook-Underwood Road	Cook, WA 98605	Robin M. Schrock
USFWS, Abernathy Salmon Culture Technology Center	1440 Abernathy Road	Longview, WA 98632	Dr. Ann Gannam
Eastern Oregon University	1410 AL≅ Avenue	La Grande, OR 97850-2899	Dr. Anna Cavinato
USGS-BRD, Marrowstone Marine Station	616 Marrowstone Point Road	Nordland, WA 98358	Nancy Elder

NPPC Program Measure Number(s) which this project addresses.

5.7A.4, 5.7B.17, 7.2A6, 7.2C, 7.2D.1, and 7.2D.3

NMFS Biological Opinion Number(s) which this project addresses.

Endangered Species Act Section 7 Biological Opinion on 1995-1998 Hatchery Operations in the Columbia River Basin, Consultation Number 383, April 5, 1995. Section VIII, Number 1 (page 66).

Other planning document references.

This proposal addresses the following action items in the National Marine Fisheries Services recommendations in their Proposed Plan for Snake River Salmon: Task 4.4.a, develop an index of measures to evaluate smolt quality and improve adult returns≅; Task 4.4.c, A...design and carry out production-scale experiments at appropriate Columbia River Basin hatcheries to test individual release strategies and evaluate smolt quality indices believed to improve smolt quality≅, and Task 4.5.b, A...release steelhead smolts that are 170 to 220 mm in total length≅.

Subbasin.

Clearwater River, Idaho

Short description.

Reduce residualism and improve smoltification of steelhead using two feed techniques: 1) enhanced diets and 2) altered feeding schedules to stimulate smoltification, reduce residualism, increase emigration success, reduce interactions with wild fish, and increase adult returns.

Section 2. Key words

Mark	Programmatic Categories	Mark	Activities	Mark	Project Types
X	Anadromous fish		Construction		Watershed
	Resident fish		O & M		Biodiversity/genetics
	Wildlife	+	Production	+	Population dynamics
	Oceans/estuaries	+	Research		Ecosystems
	Climate	X	Monitoring/eval.	+	Flow/survival
	Other		Resource mgmt		Fish disease
			Planning/admin.	X	Supplementation
			Enforcement		Wildlife habitat en-
			Acquisitions		hancement/restoration

Other keywords.

Smoltification, physiology, migration, survival, hatchery-wild interaction, smolt indices, gill sodium potassium-activated ATP-ase, diet, growth

Section 3. Relationships to other Bonneville projects

If you need more rows, press Alt-Insert from within this table.

Project #	Project title/description	Nature of relationship

Section 4. Objectives, tasks and schedules

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Increase the proportion of smolts that successfully emigrate to down river dams on the Snake and Columbia Rivers using altered feeding schedules and enhanced feeds.	1a	Monitor growth, fish health, and mortality during hatchery rearing.
		1b	PIT tag steelhead smolts prior to release.
		1c	Analyze relationship between hatchery feeding strategies and emigration success.
		1d	Interrogate PIT-tagged emigrants using the PTAGIS database system.
		1e	Prepare annual progress reports that summarize data and prepare a final project report summarizing results and findings.
2	Promote smoltification in hatchery production fish by manipulating growth rates using altered feeding schedules and enhanced feeds.	2a	Determine monthly growth rates in feed treatment groups, and determine relationship to level of smoltification, migration numbers, and rates.
		2b	Evaluate smolt condition of

			steelhead on altered and enhanced feeding during rearing and at release: monitor condition factor, mucus lysozyme, gill sodium, potassium ATPase, reflectance and total body lipids.
		2c	Determine smolt condition and health of PIT tagged migrants to determine effects of feed treatments on migration numbers and rate.
		2d	Prepare annual progress reports that summarize data and prepare a final project report summarizing results and findings.
3	Determine effects of enhanced diets and altered feeding regimes on mortality during extended seawater rearing.	3a	Transfer individuals from feed treatment groups to seawater to monitor extended seawater survival.
		3b	Monitor health and condition of fish held in seawater using necropsy based fish health assessment.
		3c	Prepare annual progress reports that summarize data and prepare a final project report summarizing results and findings.
4	Determine if enhanced diets or altered feeding schedules influence adults returns**.**	4a	Mark 20,000 fish in each treatment and control pond with coded-wire tags prior to release.
		4b	Scan all returning adult steelhead at the hatchery for coded-wire tags for tag extraction and decoding..
		4c	Analize coded-wire tag returns for statistical differences between treatment and control groups.
		4d	Prepare annual progress reports that summarize data and prepare a final project report summarizing results and findings.

5	Develop non-invasive method of total body lipid analysis for smolt monitoring purposes.	5a	Validate Short Wavelength Near Infrared spectroscopy method for the determination of total body lipids in juvenile steelhead.
		5b	Determine the relationship of growth rate, and smoltification to total body lipid content of juvenile steelhead.
		5c	Prepare annual progress reports that summarize data and prepare a final project report summarizing results and findings.

Objective schedules and costs

Objective #	Start Date mm/yyyy	End Date mm/yyyy	Cost %
1	10/1998	10/2002	40 %
2	10/1998	10/2002	30 %
3	10/1998	10/2002	10 %
4	05/2001	10/2006	10 %
5	10/1998	07/2002	10 %

Schedule constraints. .

Constraints

Flow conditions in the spring may have an affect on PIT-tag detection rates at Lower Snake and Columbia River dams.

Major Milestones

The last production release would occur in 2002. The last adult returns would occur in 2005. The final report would be completed in 2006.

Completion date. Enter the last year that the project is expected to require funding.

2002 for production research
2006 for adult return monitoring and final report completion.

Section 5. Budget

This section has two tables: 1) FY99 budget by line item, and 2) Outyear costs. Instructions for each part follow the heading.

FY99 budget by line item

List FY99 budget amounts for each category. If an item needs more explanation, provide it in the Note column. If the project uses PIT tags, include the cost ($2.90/tag). **Be sure to enter a total on the last line: this is the amount of your budget request.**

Item	Note	FY99
Personnel	Includes all the participating staff of joint sponsors	179,000
Fringe benefits		30,000
Supplies, materials, non-expendable property	Field equipment, office and lab equipment and supplies, etc	17,000
Operations & maintenance	CWT tagging (20,000 fish/pond for 18 ponds) , PIT tagging, etc.	32,000
Capital acquisitions or improvements (e.g. land, buildings, major equip.)	Desktop Computer, software, digital camera	3,500
PIT tags	# of tags: 7200	21,000
Travel		11,000
Indirect costs		94,700
Subcontracts		
Other		
TOTAL		388,200

Outyear costs

List budget amounts for the next four years, and the estimated percentage of those costs for operations and maintenance (O&M).

Outyear costs	FY2000	FY01	FY02	FY03
Total budget	388,200	388,200	356,000	195,000
O&M as % of total	8%	8%	0%	0%

Section 6. Abstract

Reducing residualism for summer steelhead at Dworshak NFH will be addressed in a production level research project conducted jointly by the USFWS, USGS-BRD, and Eastern Oregon University. The project addresses key components of the 1994 Columbia Basin Fish and Wildlife plan that regard steelhead populations, specifically measures 5.7A.4, 5.7B.17, 7.2A.6,

7.2C, 7.2D.1, and 7.2D.3.

The project was designed to evaluate new feeding strategies to stimulate smoltification over a wider range of fish sizes thereby reducing residualism and precocity. Manipulation of growth rates and smolt physiology will be effected by changes in hatchery feeding practices. New feeding strategies, that include an altered feeding schedule and two enhanced feeds, will be evaluated at the production level by monitoring growth, survival, emigration success, and adult returns of treatment groups compared to regular production control groups. Physiological development, smolt condition and health will be monitored during rearing, emigration, and extended seawater holding. Improved physiological condition, enhanced smoltification, increased emigration success, prolonged seawater survival, and increased adult returns will determine the effectiveness of the different feeding strategies.

Section 7. Project description

a. Technical and/or scientific background.

The Dworshak National Fish Hatchery stock is characterized by a wide length frequency distribution at the time of release. This does not conform to the Biological Opinion from the National Marine Fisheries Service (NMFS) on Hatchery Operations for 1996-1999 that calls for hatchery steelhead to be released between 170-220 mm (TL) in order to minimize residualization. The Draft Snake River Salmon Recovery Plan (SRSRP) recognizes that ASteelhead larger than 170 mm experience more complete parr-smolt transformation and are therefore more likely to actively migrate≅. Fish larger than 220 mm are more prone to residualize (Partridge 1985, 1986; Cannamela 1992) and steelhead greater than 250 mm may be more capable of predation (Cannamela 1993).

Wide length frequency distributions are a common characteristic of juvenile steelhead at Dworshak NFH. Lengths often range from 80 to 240 mm (total length) by the time they are released as smolts. Research conducted by the Idaho Fishery Resource Office and Dworshak NFH indicates that a high proportion, as many as 25%, of the steelhead that are less than 170 mm at the time of release may not be migrating downriver to the ocean (Bigelow 1995; Bigelow 1997). Although a high proportion (about 50%) of steelhead less than 170 mm apparently fail to outmigrate, the remaining proportion <170 mm do outmigrate. Wild steelhead smolts outmigrating from the Snake River basin are generally smaller on the average than their hatchery counterparts. Many wild salmon stocks exhibit osmoregulatory competence at sizes much smaller than hatchery stocks (Zaporozhec and Zaporozhec 1993; Shrimpton *et al.* 1994). Naturally spawning stocks outmigrating from Columbia River tributaries exhibit a wide range of lengths and ages due to the harsh environment (Peven *et al.* 1994), providing further evidence that size alone does not control smoltification.

Of particular concern is the potential interaction of residual steelhead with sensitive species such as the endangered Snake River fall chinook, spring and summer chinook, or the proposed, as threatened, wild Snake River steelhead. Interactions include displacement, competition for food, and behavioral effects (Viola and Schuck 1995; McMichael *et al.* 1997). The primary objective of this project is to manipulate growth and smoltification to minimize the number of non-migrating hatchery summer steelhead released into the Snake River basin.

Hatchery managers may develop production strategies that produce steelhead that meet the SRSRP guidelines for size at release, but that does not necessarily insure an increase in the proportion of actively migrating steelhead smolts released. Hatchery practices can have a significant influence on the parr-smolt transformation process (Folmar and Dickoff 1980; Shrimpton *et al*. 1994), and need to be developed based on a knowledge of how they directly influence the growth, physiology, and behavior of steelhead leading up to and during smoltification. Previous studies to reduce growth in steelhead did not address the effects of changes in growth rate on smolt condition (Smith 1987; Klontz *et al*. 1991). Research with chinook salmon found that both the size of ration and feeding schedule may influence the peak of gill sodium, potassium-activated ATPase (Ewing *et al*. 1980).

We address the residualism issue from a completely different perspective, promoting the idea that the real need is to produce healthy, high quality, actively migrating steelhead smolts, regardless of size. It is a natural phenomenon in the life history of steelhead stocks in the Columbia River to exhibit a wide range of sizes and ages at the time of smolt outmigration (Peven *et al.* 1994). A review of the literature indicates that high variability in size (length) within a juvenile steelhead population is in part a product of social interactions, and the establishment of dominant and subordinate individuals, where dominants grow significantly faster than subordinates (Abbott and Dill 1989). This same phenomenon has been observed in Atlantic salmon populations (Symons 1970; Thorpe *et al.* 1992). Subordinate individuals in a population generally experience slower growth rates and higher levels of stress than dominant individuals. It is our contention that this situation leads to arrested development of subordinate individuals, and a failure to make the successful transformation from parr to smolt. Further literature review indicates that growth rate at the time of smoltification may be more critical in the parr-smolt transformation process than the absolute amount of growth that occurs (Whitesel 1991).

We propose to remedy the wide length frequency distribution in steelhead by changing growth and smoltification patterns using a combination of two cultural techniques: 1) enhanced diets formulated to facilitate or stimulate the physiological processes that occur during smoltification, and 2) manipulation of growth rates prior to and during smoltification using a modified feeding strategy.

The underlying assumption of this project is that manipulation of feeding strategies during rearing will result in changes in growth during the parr-smolt transformation that will prompt more fish to migrate by promoting smoltification in a larger proportion of the production. Present guidelines assume that fish length is the primary variable responsible for successful smoltification. Evidence does indeed suggests that there is a critical size threshold that is important in the timing of seaward migration (Folmar and Dickoff 1980; Whitesel 1991). For steelhead, research indicates that salinity preference can be size and age dependent (Folmar and Dickoff 1980). Conte and Wagner (1965) concluded that steelhead less than 120 mm had not reached the critical or optimal size to have developed an effective osmotic and ionic regulating system. Their data suggests that the critical size for steelhead is about 140 to 150 mm.

Rearing practices within the hatchery can affect the onset of smoltification as well as the process of smoltification itself, and can have dramatic effects on the success of the parr-smolt transformation (Folmar and Dickoff 1980; Hansen *et al.* 1988). Therefore, to focus attention primarily on fish size alone disregards the complex interaction between growth and

smoltification, and the need to develop a physiological approach for insuring a higher parr-smolt transformation rate for hatchery steelhead.

Accelerated growth and smoltification may be induced by photoperiod and temperature manipulation during experiments (see Hansen *et al*. 1989) , but managing these variables at a large production hatchery is difficult. Altering hatchery feeding regimes and diets may offer a management alternative for improving production. Low levels of ration have been found to alter feeding behavior (Dill 1983) and may provide a strategy to influence the social hierarchy in a hatchery population. Smaller daily rations have been shown to produce growth rates equal to rates with higher daily rations by improving feeding efficiency (Farmer *et al.* 1983). Ration levels have a pronounced effect on growth rates, but the rate of increase of growth rate and gross feed conversion efficiency decreases with increased rations (McCormick *et al.* 1989). Feeding rates ranging from 1 - 5 % body weight did not affect the physiological quality of hatchery reared chum salmon (Ban *et al.* 1995). We propose to stimulate the onset of smoltification over a wider range of the population by sustaining a lower growth rate prior to the time the fish are known to smolt.

Feed, growth, and adult return records from the past ten years have been analyzed to determine the reduction in feed needed to attain minimum growth rates comparable to those in years of high adult returns when small individuals were released (Schrock, personal communication). The growth rate will then be accelerated during a critical period from February to the time of release to induce smoltification. The objective is to produce fewer large individuals that residualize, while increasing the growth rate will help to initiate smoltification in the smaller individuals in the population. Two other feed formulations will be compared to the hatchery production feed. All three of these diets, the steelhead production, Abernathy, and Moore-Clarke diets are all considered high protein (> 45%) and high fat (> 14%) diet . The Abernathy is an open formula diet, while the steelhead diet and Nutra Transfer diet are closed formula, with proximate analysis results being available from the manufacturers (Rangen and Moore-Clark, respectively). High protein (up to 47%) increases growth rate and feed efficiency. Fat (up to 14%) also improves feed efficiency. However, Akiyama et al. (1981) observed that feeding rate in chum salmon may drop with improved feed efficiency. Nutra Transfer Diet has a much higher fat content (proximate analysis 25%) than the others. Dietary fat influences both growth and smolting (Ogata and Konno 1989; Ogata and Murai 1989) in high protein diets, but levels of fat as high as 25% have not been tested. Lipid metabolism and changes in the fatty acid composition are known to change during smoltification in steelhead trout (Sheridan et al. 1985, Sheridan 1988).

Immune enhancers are a component of both the Abernathy and Moore-Clarke feeds. Glucans have been found to enhance disease resistance and survival in number of salmon species (Robertsen *et al.* 1994). Lysozyme activity was increased in Atlantic salmon (*Salmo salar*) and rainbow trout (Engstad et al. 1992, Jorgansen et al. 1993) administered glucan. In rainbow trout fed several different immunostimulants, there was in increase in oxidative radical release, myeloperoxidase activity, phagocytic indexes, and potential killing activities of phagocytic cells including neutrophils (Siwicki *et al.* 1994). The purpose of the glucan additive is to improve the health and survival of production fish during rearing and migration. The Moore-Clarke feed contains salt, an additive that has been found to increase seawater adaptability of non-smolting rainbow trout (Salman and Eddy 1990).

Smolt condition will be evaluated using physiological indices that have characterized DNFH

steelhead in earlier studies to allow comparison among past years. The methods include condition factor; gill sodium, potassium ATPase (Schrock *et al.* 1994); reflectance Haner *et al.* 1995); mucus lysozyme (Schrock 1994); and total body lipids (Cavinato *et al.*1996, AOAC 1997). The methods will allow for comparison of physiological develop in the experimental groups with production steelhead from Dworshak, and elsewhere, under different rearing regimes.

Snake River steelhead have been listed as threatened under ESA, therefore new non-invasive methods to determine total body lipids will be validated for steelhead to allow comparison of production fish with wild steelhead. The Short Wavelength Near Infrared (SW-NIR) spectroscopy method will be validated against a conventional extraction method currently used in fisheries research (Sheridan 1983; AOAC 1997). Proximate analysis of fish tissues have been reported in near-infrared (Gjerde and Martens 1987; Rasco et al. 1991). The desired outcome is to document growth patterns, and lipid deposition and depletion during rearing, smoltification, and the migration.

Extended seawater survival will be monitored at Marrowstone Marine Station (USGS-BRD). Fish will be transported at the time of release, acclimated to seawater over 4 days, and inventoried on a monthly basis for growth, survival, and health status by necropsy based assessment.

The proposed projects addresses three important regional management documents and their plans for steelhead in the Columbia and Snake Rivers. The combined specialization and effort of four facilities in the region has produced a production level research design to determine a method of reducing residualism in hatchery steelhead.

b. Proposal objectives.

Objective 1. Increase the proportion of smolts that successfully emigrate to down river dams on the Snake and Columbia Rivers using altered feeding schedules and enhanced feeds.
Null Hypothesis: Steelhead populations fed enhanced diets or using altered feeding schedules do not have a higher proportion of actively migrating smolts than regular production populations of steelhead.
Assumptions: a) Rearing conditions and practices at the hatchery are identical for all treatment and control groups. b) Treatment and control groups are interrogated at the dams in the same proportions.
Products: Annual reports will provide summaries of growth, mortality, and fish health during hatchery rearing; summaries of migration rates and interrogation rates at Lower Snake River and Columbia River dam; comparisons of residualism rates between treatment and control groups. A final report will provide compilation and summary of data for all three years including statistical analysis that will describe relationships between treatments and smoltification.

Objective 2. Promote smoltification in hatchery production fish by manipulating growth rates using altered feeding schedules and enhanced feeds.
Null Hypothesis: There is no difference in smolt condition between steelhead populations fed enhanced diets using altered feeding schedules or enhanced feeds and regular production populations of steelhead. The null hypothesis will be tested for all individual physiological

measurements.
Assumptions: a) Rearing conditions are identical for all treatment and control groups.
b) Treatment and control groups are interrogated at the dams in the same proportions.
Products: Multivariate analysis will be used to analyze relationships among smolt indices in steelhead reared under the different feeding strategies. Annual reports will provide summaries of smolt conditions and physiology during hatchery rearing and after emigration to the dams. A final report will provide compilation and summary of data for all three years including a statistical analysis that will describe relationships between treatments and smoltification.

Objective 3. Determine effects of enhanced diets and altered feeding regimes on mortality during extended seawater rearing.
Null Hypothesis: Steelhead populations fed enhanced diets or using altered feeding schedules do not have a higher rate of survival during extended seawater rearing trials than regular production populations of steelhead.
Assumptions: Rearing conditions and practices at the hatchery are identical for all treatment and control groups.
Products: Annual reports will provide summaries of mortality rates for treatment and control group. The final report will compile all data and provide a statistical analysis of mortality during seawater rearing.

Objective 4. Determine if enhanced diets or altered feeding schedules influence adults returns**.**
Null Hypothesis: Steelhead populations fed enhanced diets or using altered feeding schedules do not have higher smolt to adult return rates.
Assumptions: Rearing conditions and practices at the hatchery are identical for all treatment and control groups.
Products: Annual reports will provide summaries of adult returns and comparisons between treatment and control groups using coded-wire tag returns; summaries of smolt to adult return rates will be provided. A final report will provide compilation and summary of data for all three years including a statistical analys that will describe relationships between treatments and smoltification.

5. Develop non-invasive method of total body lipid analysis for smolt monitoring purposes.
Null Hypothesis: There is no difference in total body lipids between production steelhead and steelhead fed on an altered feeding schedule or enhanced feeds.
Assumptions: a) Measurements of total body lipids by short wavelength near infrared spectroscopy will correlate with extracted total body lipid measurements in steelhead, as they have in other species. b) Rearing conditions and practices at the hatchery are identical for all treatment and control groups.
Products: Performance will be measured by the correlation of total body lipids as measured by Short Wavelength Near Infrared Spectroscopy with total body lipids determined by extraction and wet chemistry analysis (AOAC, 1997). Results will be included in the annual and final reports. The method will be published in a peer reviewed article.

c. Rationale and significance to Regional Programs.

The proposal specifically addresses elements of the Draft Snake River Salmon Recovery Plan (SRSRP) and the Biological Opinion from the National Marine Fisheries Service (NMFS) on Hatchery Operations for 1996-1999 that call for hatchery steelhead to be released in a specific size range to minimize residualization. The project objective, to reduce residualism in production steelhead, tests the hypothesis that length is the sole indicator of smoltification and predictor of migration success. Project sponsors from the USFWS, USGS-BRD, and a state university have combined their expertise in production, nutrition, smolt physiology, and diagnostic chemistry to design an experiment that tests the effects of changes in hatchery feeding practices on physiological development leading to smoltification. Furthermore, interactions with wild populations would be reduced if residualism were reduced and emigration success was increased. The proposal is a result of previous cooperation among the joint sponsors on a variety of projects that includes smolt monitoring, fish health and nutrition issues, and information exchange. A critical element of the proposal is that no single sponsor could carry out the project without the assistance of the others.

The project was first proposed by the Idaho Fishery Resource Office, who will coordinate project activities. The Abernathy Salmon Culture Technology Center has produced a special feed for the study, and a commercial feed producer, Moore-Clarke, has offered a second feed for testing. USGS-BRD staff developed the research design with the IFRO, provided data analysis to determine the altered feeding regime for Dworshak, and will conduct the smolt monitoring necessary to evaluate the test groups. USGS-BRD staff is participating in the validation of the non-invasive total lipid analysis with EOU. EOU staff will participate in physiological sampling during the experiment, and will develop the final protocol for Short Wavelength Near Infrared (SW-NIR) spectroscopy for total body lipids for steelhead. The method will provide a novel non-invasive technique for future studies to compare growth patterns in wild and hatchery steelhead for a better understanding of smoltification. The project developed from the association of staff of the Assessment of Smolt Condition for Travel Time Analysis project (USGS-BRD) with Dworshak National Fish Hatchery during its monitoring project at Dworshak National Fish Hatchery, 1988-1996. Cooperative studies between Abernathy Salmon Culture Technology Center and the Columbia River Research Laboratory have explored the effects of glucan feed enhancement and disease resistance (Schrock and Gannam, unpublished data). Marrowstone Marine Station has held Dworshak steelhead for extended seawater survival in previous experiments. Eastern Oregon University has investigated Short Wavelength Near Infrared (SW-NIR) spectroscopy in rainbow trout and chinook salmon at commercial and state hatcheries (Cavinato 1996, Songprachakkul 1997, Rasco 1997).

The proposed production project is designed to reduce residualism in mitigation steelhead in the Clearwater River, Idaho in direct response to elements of the FWP, NMFS Biological Opinion regarding hatchery operations, and the Snake River Steelhead Recovery Plan. It is a carefully organized project involving multiple agencies and facilities that addresses the need on a production level.

d. Project history (for continuing projects).

Not Applicable

e. Methods.

Objective 1. Increase the proportion of smolts that successfully emigrate to down river dams on the Snake and Columbia Rivers using altered feeding schedules and enhanced feeds.

Tasks: Groups of summer steelhead at Dworshak NFH will be set up in outside rearing ponds so that each of three treatment and control groups have at least three replicate ponds. Treatments will consist of: a) altered feeding schedule designed reduce growth from December 1 to February 15, followed by a normal feed schedule with feed augmentation through time of release. b) feeding an enhanced diet from Moore-Clarke for six weeks prior to the date of release; c) feeding an enhanced diet from Abernathy Salmon Culture Technology Center for six weeks in the spring prior to release. Two weeks prior to release, 200 fish above and below 170 mm (TL) from each pond will be PIT tagged. After smolts are released, PIT-tag interrogation data from Lower Snake and Columbia River dams will be downloaded from the PTAGIS database for each of the treatment and control groups.

Data Analysis: 1) PIT-tag data will be compiled into standard databases for statistical summary and analysis. Migration rates will be compared between treatments and controls using a standard T-Test. Survival will be calculated using a Jolly Seber estimate.

Expected Results: We expect to determine whether enhanced feeds and altered feeding regimes significantly increases the proportion of steelhead smolts that successfully emigrate downriver to the ocean. Replication of the experiment will involve three production years.

Objective 2: Promote smoltification in steelhead by manipulating growth rates using altered feeding schedules and enhanced feeds.

Task 2a: Monitoring of growth rates of control and treatment groups will occur during rearing and before release, and will be related to the physiological measurements made at the same time. Multivariate analysis will be applied to test for relationships among all variables measured.
Task 2b: Smoltification will be monitored using health assessment by the DFHL, gill sodium, potassium ATPase (Schrock *et al.*1994); reflectance (Haner *et al.* 1995), mucus lysozyme (Litwack 1955, Muona and Soivio 1992), and total body fat analysis by SW-NIR(Cavinato 1996, Songprachakkul 1997, Rasco 1997) and lipid extraction (AOAC 1997) with moisture analysis (Rasco 1991). Sample size has been established for the physiological measurements by statistical analysis of test group results to determine the minimum sample size necessary to distinguish among treatment groups. A minimum of 40 fish must be sampled per group to detect difference among test groups for skin mucus lysozyme, while power analysis of other indices suggests smaller samples sizes.
Task 2c: Physiological monitoring of all PIT tagged individuals will continue at lower Snake Rivers Dams to establish correlations among growth rates and smoltification indices.

Data Analysis: Multivariate analysis will be applied to all smolt condition data collected to

determine differences among the treatment groups.

Expected Results: All fish will be raised in standard production ponds at DNFH. The critical assumptions are that feeding methods affect growth rate, that growth rate affects smolt condition, and that smolt condition determines migration numbers and rates. It is expected that there will differences in growth rate, smolt condition, and migration success among the treatment groups.

Objective 3: Determine effects of enhanced diets and altered feeding regimes on mortality during extended seawater rearing.

Tasks: Performance of feed strategy and production controls will be monitored during prolonged seawater holding at Marrowstone Marine Station. Periodic inventory will compare survival, condition factor, and health by necropsy based fish health assessment among the treatment groups.

Data Analysis: Multivariate analysis will determine the significance of differences among treatment groups during seawater rearing.

Expected Results: A critical assumption is that different feeding strategies result in differences in smolt condition and seawater adaptability that will be detected as differences in seawater survival among the treatment groups.

Objective 4: Determine if enhanced diets or altered feeding schedules influence adults returns.

Tasks: In the fall prior to release, we will mark 20,000 fish in each of the treatment and control ponds with code-wire tags to determine treatment effects on adult returns. Prior to release, estimates of coded-wire tag retention will be made by taking random samples from each pond. All adults returning to the hatchery will be scanned for tags which will be extracted and read.

Data Analysis: Mean rates of adult returns will be calculated for each treatment and control group. Means will be tested for significant differences using a standard T-Test.

Expected Results: We expect to determine whether feeding enhanced feeds or using altered feeding schedules significantly increases the number of adults that return.

Objective 5: Development of a non-invasive measurement for total body lipids.

Task 5a: Short Wavelength Near Infrared (SW-NIR) spectroscopy method for total body lipids will be validated against a standard extraction and wet chemical method (AOAC 1997).
Task 5b. Total body lipid measurements made during rearing, at release, and during the migration will be compared by multivariate analysis to the other smolt indices. A critical assumption is that differences in growth rate and smoltification will be detected as differences in total body lipids among the feed treatment groups. It is expected that non-invasive total body

lipid analysis by SW-NIR spectroscopy will prove a useful method of monitoring growth in juvenile steelhead.

Data Analysis: Total body lipid measurements by chemical and SW-NIR spectrometric analysis will be analyzed using Partial Least Squares (PLS) to establish the calibration model. Total body lipid measurements will be included in multivariate analysis to determine differences among treatment groups.

Expected Results: It is expected that differences in growth rates and condition among treatment groups will be detected as differences in total body lipids among treatment groups.

f. Facilities and equipment.

Dworshak Fishery Complex

The fish rearing and treatment application will be conducted at the Dworshak Fishery Complex at Orofino, Idaho, which includes the Dworshak NFH, the Idaho Fishery Resource Office, and the Dworshak Fish Health Center. Currently, Dworshak NFH raises and releases about 2.3 million summer steelhead annually and has 85 Burrows ponds for steelhead rearing. The Fishery Resource Office has conducted research and evaluation studies for chinook salmon and steelhead at this facility for nearly 12 years. Sufficient facilities and equipment are presently available to accommodate the project. Office and laboratory space is available for conducting all necessary activities.

Columbia River Research Laboratory

The facility offers 1600 sq. ft. of analytical laboratory space including biochemistry, physiology, and immunology labs. A 1500 sq. ft. wet lab with 64 tanks is serviced with river and well water. Field technology capabilities employee 25 vessels geared for radio telemetry, hydro acoustics, electroshock, diving, and in-river sampling.

Abernathy Salmon Culture Technology Center

The facility, with 12 raceways, 120 tanks, and 50 egg incubators offers large scale research opportunities. Water sources include direct stream or well water, and can supply 3500 gpm of pathogen free water at 12oC. Chemistry and microbiology laboratories, and a feed extruder for salmon `life-stage' diet development provide a unique capability to conduct complete life history studies for salmon, steelhead, and sturgeon.

Marrowstone Marine Station

Located on Marrowstone Island on northern Puget Sound, the newly remodeled and expanded marine facility has 9456 sq. ft. of wet lab space equipped for fish holding, and a fully equipped analytical laboratory. High quality seawater is available for seawater holding. The station provides cooperators with support for projects examining seawater survival of anadromous salmon, disease and stress in salmon, contamination in marines species, and marine species rearing technology.

Eastern Oregon University

Eastern Oregon University is located in LaGrande, Oregon. The Chemistry Department offers 2798 sq. ft. of laboratory space. The department offers specialized analytical capabilities including UV-VIS and SW-NIR spectroscopy, Gas Chromatography, HPLC, AA and NMR technology. Fishery research at EOU is funded by the Oregon Sea Grant , and EOU chemists work in cooperation with Oregon Department of Fish and Wildlife biologists.

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Section 8. Relationships to other projects

The proposed project complements the programs of all participants by allowing expanded research potential by pooling the expertise, methods, personnel, and facilities of the sponsoring agencies. A production level research project with a scope of activities that could not be implemented solely by any one of the participants has resulted. This project was developed by personnel from different facilities and agencies that share information at meetings and on small, cooperative projects that do not depend on funding from a single source. Dworshak National Fish Hatchery served as one of the reference hatcheries for steelhead for the Assessment of Smolt Condition for Travel Time Analysis project from 1988-1993. Personnel from the USFWS, ASCTC and the USGS-BRD, CRRL have cooperated on studies to test the effects of glucan in feed on disease resistance. DNFH has transported spring chinook at the USGS, Marrowstone Marine Station to test extended seawater survival. The USGS-BRD, CRRL has provided steelhead to EOU for preliminary investigations of total body lipids in steelhead. There are no known permitting or conflicting demands known.

Section 9. Key personnel

Principal Investigator: Ray N. Jones, Fishery Biologist
Idaho Fishery Resource Office, U.S. Fish and Wildlife Service

Smolt physiologist: Robin Schrock, Research Fishery Biologist
Columbia River Research Laboratory, USGS - Biological Resources Division

Advising fish nutritionist: Dr. Ann Gannam, Nutritionist
Abernathy Salmon culture Technology Center, U.S. Fish and Wildlife Service

Marine facility, Acting Director: Nancy Elder, Fishery Biologist
Marrowstone Marine Station, USGS - Biological Resources Division

Chemist: Dr. Anna Cavinato, Assistant Professor of Chemistry
Eastern Oregon University

Ray N. Jones

EDUCATION

Master of Science, Zoology - Oklahoma State University **1981**
Major: Fisheries Ecology Stillwater, Oklahoma

Bachelor of Science, Fisheries Resources - University of Idaho **1977**
Major: Fisheries Management Moscow, Idaho

EMPLOYMENT

Fishery Biologist **1991 to Present**
U.S. Fish and Wildlife Service Ahsahka, Idaho
Responsible for conduction hatchery evaluations at Dworshak and Kooskia NFHs; Identifies contraints in hatchery production and designs and conducts research projects to improve production practices; Team Leader for both the Dworshak and Kooskia Hatchery Evaluation Teams.

Fishery Biologist **1986 to 1991**
U.S. Fish and Wildlife Service Kenai, Alaska
Developed and conducted fishery resource investigation projects on National Wildlife Refuges. Developed Refuge fishery management plans.

Fishery Biologist **1983 to 1986**
Nez Perce Tribe Department of Fishery Resources Lapwai, Idaho
As the Departments Harvest Manager, Jones was responsible for planning and developing harvest management plans and collecting fishery harvest information. Jones participated on various interagency groups to prepare comprehensive enhancement plans for the Columbia River.

EXPERTISE

Jones has worked nearly 14 years as a fishery biologist. The past 6 years has been at the Idaho FRO assisting the staffs at Dworshak and Kooskia NFHs conduct evaluation projects to improve steelhead and spring chinook production. Jones provides the leadership for the Hatchery Evaluation Teams at both of these hatcheries. This experience well qualifies Jones for coordinating the activities of the Joint Sponsors of this proposal.

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Robin M. Schrock

Education

M.S., University of Wisconsin, Stevens Point, WI (Natural Resource Management - Fisheries) 1986
B.A., Portland State University, Portland, OR (Biology and German) 1982
Diploma, School for Medical Technologists, Bern, Switzerland 1975

Experience

Research Fishery Biologist, USGS-Biological Resources Division,
Columbia River Research Laboratory 1991 to present
Fishery Biologist, USFWS-National Fishery Research Center
Marrowstone Field Station 1987-1991

Publications and Reports

Schrock, R.M., J.W. Beeman, D.W. Rondorf, and P.V. Haner. 1994. A microassay for gill sodium, potassium-activated ATPase in juvenile Pacific salmonids. Transactions of the American Fisheries Society.

Haner, P.V., J.C. Faler, R.M. Schrock, D.W. Rondorf, and A.G. Maule. 1995. Skin reflectance as a non-lethal measure of smoltification for juvenile salmonids. North American Journal of Fisheries Management 15:814-822.

Maule, A.G., R.M. Schrock, C. Slater, M.S. Fitzpatrick, and C.B. Schreck. 1996. Immune and endocrine responses of adult spring chinook salmon during freshwater migration and sexual maturation. Fish and Shellfish Immunology 6:221-233.

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Ann L. Gannam

Education

Ph.D., Auburn University, Auburn, Alabama (Fish Nutrition /Aquaculture) 1988
M.S., University of Southern Mississippi, Hattiesburg, Mississippi (Biology) 1980
B.S., University of Georgia, Athens, Georgia (Zoology) 1976

Experience

USFWS, Abernathy Salmon Culture Technology Center. Nutritionist, 1992-present
New diet development, quality control inspections of feed mills, feeding trials.
Assistant Professor, Fisheries, University of Arkansas - Pine Bluff, Arkansas 1989-1992
Department of Agriculture. Taught fisheries courses, conducted fish nutrition research.
Adjunct assistant professor at the University of Arkansas Fayetteville. 1988-1989

Presentations

Schrock, R. M. and A. Gannam. 1996. Comparison of three glucan preparations as feed additives in juvenile fall chinook salmon (Oncorhynchus tshawytscha) challenged with Vibrio anguillarum. Presented at the American Fisheries Society Fish Health Section Meeting, Madison, WI, August 6-9 1996.

Gannam, A. L., R. M. Schrock and M. W. Hack. 1997. The use of three glucan preparations as feed additives in diets for fall chinook salmon, Oncorhynchus tshawytscha. Poster presentation at the World Aquaculture Meeting, Seattle, WA, February 19-23 1997.

Gannam, A. L. 1997. Development of open formula diets and new feeding strategies: A progress report. 48th Annual Pacific Northwest Fish Culture Conference, Gleneden Beach, OR, December 2-4, 1997.

Nancy Elder

Education
B.S., Purdue University, West Lafayette, IN (Animal Science) 1980

Experience
USGS-BRD, Marrowstone Marine Station, Acting Director 1995 to present
NBS (formerly USFWS), Marrowstone Field Station, Fishery Biologist 1987-present
University of Idaho, Research Associate - Fisheries Dept. 1985-1987

Anna G. Cavinato

Education
Ph.D. Universita' degli Studi di Bari, Bari, Italy (chemistry) 1981

Experience
Eastern Oregon University, Assistant Professor of Chemistry. 1992 to present
University of Washington, Research Assistant Professor, 1988-1992.
Memphis State University, Research Associate, 1986-1988.
University of Tennessee at Memphis, Research Associate, 1984-1986.
University of Tennessee at Knoxville, Research Associate, 1983-1984.

Publications
Cavinato, A.G., D.M. Mayes, Z. Ge, and J.B. Callis. 1990. A non-invasive method for monitoring ethanol in fermentation processes using fiber-optic-near-infrared spectroscopy. Analytical Chemistry 62:1977-1982

Clark, M.M., A.G. Cavinato, D.M. Mayes, B.A. Rasco, and Y. Sin. 1997. Non-invasive SW-NIR spectrophotometric method for determination of lipid content in muscle of rainbow trout. Eastern Oregon Science Journal XIII:9-14.

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Mayes, D.M., A.G. Cavinato, and D.S. Koza. 1993. A compact solid state spectrophotometer system for control applications. Process Control and Quality 5:1-8.

Section 10. Information/technology transfer

Results of the project will be published in an acceptable peer reviewed scientific journal. Data from PIT-tagging will become incorporated into the PTAGIS database. Coded-wire tag data will be incorporated into U.S. Fish and Wildlife Service databases. Physiological data will be incorporated into the Assessment of Smolt Condition for Travel Time Analysis comprehensive database. Presentations will be made at regional and national workshops, conferences, and symposiums.